Fabrication of Hydrogel Using Mixture of Seaweed Biopolymers and to Evaluate Its Biological and Mechanical Properties

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Abstract
Aim: The aim of the study is to estimate the biological and mechanical properties of hydrogel fabricated from the mixture of sea weeds Introduction: Hydrogel is a promising material for local antimicrobial application. Hydrogel refers to a kind of biomaterial synthesized by either synthetic or biopolymer. Carrageenan is used for coughs, bronchitis, tuberculosis, and intestinal problems. The ability to combine agar with chitosan and carrageenan with inorganic substances such as tricalcium phosphates and hydroxyapatite makes these matrices also useful for oral delivery, burn wound healing and bone TE. Chitosan has potent, much adhesion, homeostatic action, film-forming ability, biodegradability and anti-microbial action. Materials and Methods: In a flask 0.1g of chitosan is added to the distilled water and heated for an hour. Meanwhile the solubility is checked. After checking the solubility of 0.1g of Agar, Rutin and 0.2g of carrageenan added to 100 ml of chitosan. Mixed solution is kept Ina magnetisms stirrer for 1 hour at 100 degrees Celsius. Anti-microbial activity : Agar well diffusion and Disc diffusion method is used. Then incubated for 37 degrees Celsius for 48 hours. The zone of inhibition is recorded. Mechanical properties: (FTIR) Fourier transform infrared and contact angle were measuredRESULTS And Discussion: The results showed that the hydrogel formed from the mixture of seaweed has potential antimicrobial activity against streptococcus mutans as the concentration increases and it also showed that the physical properties of the hydrogel derived from seaweed had more promising characteristics as the concentration increases.Conclusion: Seaweed will provide striking bioactives, which can be used as a promising medicine for the treatment of human diseases, orelse it can be used as new antimicrobial agents to be the replacement for synthetic antibacterial agents used in various applications. Hydrogel prepared from the mixture of seaweeds can be used in the application of drug delivery; it had shown that it had promising antimicrobial activity and physical properties.
Keywords: Periodontal tissue engineering, hydrogels, Rutin, Carrageenan, Hydrogel, FTIR, Agar.

1. Introduction

Complete and predictable regeneration of periodontal tissues lost due to disease has always been the main motive behind periodontal therapy(1). Though various treatment modalities and materials are available, the complete and predictable regeneration of periodontal tissues has still been elusive(2). Various non-surgical modalities like mechanical debridement, chemotherapeutics and periodontal flap surgeries aim at removing the etiologic agents and arresting the active tissue destruction(3). Guided tissue regeneration along with bone grafting are the gold standard treatment for correction of bony defects and periodontal regeneration(4). However, various factors like the technique sensitivity, bioactivity of materials, skill of the surgeon affect the regenerative capacity of these materials. Application of periodontal tissue engineering approaches have emerged as the solution for the complete and predictable regeneration of tissues, where in cells, scaffolds and growth factors are applied (5). Our team has extensive knowledge and research experience that has translated into high quality publications (6–15). Hydrogels have recently attracted a lot of interest in the realm of regenerative medicine. Hydrogels are three-dimensional water-swollen polymeric

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materials that are widely employed in biomedical applications like cell culture, drug administration, and tissue engineering. They offer excellent biocompatibility, mechanical strength, and accessibility (16)(17). Their physical properties like high porosity three dimensional structure with excellent hydrophilic ability and adjustable viscoelastic behavior, mimic the extracellular matrix that is favorable for cellular attachment, proliferation and differentiation(18). Hydrogels can be customized according to requirements by addition of drugs, growth factors and cells of interest (19).

Classification Of Hydrogels

Based On Source
Natural
Synthetic
Based On Polymeric Composition
Homopolymeric
Copolymeric
Multipolymeric
Based On Type of Crosslinking
Chemical
Physical
Based On Configuration
Crystalline
Semicrystalline
Amorphous
Based On Network Electric Charge
Nonionic
Ionic
Amphoteric
Zwitterionic

Hydrogels For Periodontal Regeneration

Hydrogels can be broadly classified based on their sources into natural and synthetic. Natural hydrogels are obtained from natural products like marine seaweeds and are mainly composed of natural polymers, glycosaminoglycans, polysaccharides, proteins that make them mimic the extracellular matrix composition. They are highly hydrophilic biocompatible but exhibit poor mechanical strength. Natural hydrogels are mainly composed of chitosan, collagen, carrageenan, alginates etc. various physical and chemical crosslinkers are added to improve the mechanical strength of natural hydrogels. Synthetic polymers are prepared through physical and chemical crosslinking. Common synthetic compounds, such as polyethylene glycol (PEG) , PVA , and poly(lactic-co-glycolic acid) (PLGA) have good mechanical strength and stability but they lack bioactivity. Their biocompatibility is also poor when compared to natural hydrogels.

Hydrogels have multiple applications in periodontal therapy. They can be used as drug carriers with slow and sustained release (local drug delivery), biomimetic scaffolds for growth factors, cells and self-healing hydrogels. Recently, intelligent hydrogels have been developed that respond to the changes in the external environment ex thermosensitive, photosensitive and pH-responsive hydrogels.

In the present study, we prepared a hydrogel for periodontal tissue engineering application with carrageenan, chitosan, rutin and analyzed its antimicrobial and mechanical properties.

2. Material And Methods

The study was conducted at the Department of Biomaterials at Saveetha Dental College, Chennai. The hydrogel was prepared using carrageenan, agar, chitosan and rutin as the components.

Source Of Biomaterials

Carrageenan, agar, chitosan and rutin were obtained from Himedia Laboratories Pvt Ltd , Mumbai, India. Chitosan from shrimp cells with molecular weight 3800 - 20000 Daltons and 75 % degree of deacetylation was used. Rutin powder (Nature's harvest) was obtained from PC Industries.

Fabrication Of Hydrogel

The injectable hydrogel was prepared with agar(0.1%), carrageenan(0.1%), chitosan(0.1%) and rutin (0.1%) as components. The chitosan extracted from the shrimp shells with a high molecular weight of 3800-20,000 daltons was used. Chitosan was dissolved in 0.1% acetic acid. Distilled water was added to the mixture to obtain the required concentration. The mixture was then stirred at 65 degrees and 500 rpm for 20 minutes. Agar, carrageenan were then added to this mixture, stirred and allowed to mix thoroughly for 2 hours. Rutin was added to the entire components to obtain a hydrogel consistency. The prepared hydrogel was then subjected to antimicrobial analysis, Contact angle analysis and FTIR.

Antimicrobial Testing

Zone of inhibition (ZOI) measurement

Antimicrobial activity of the seaweed derived hydrogel were assessed against streptococcus mutans, staphylococcus aureus and candida albicans using Mueller–Hinton agar (MHA; Himedia, Mumbai, India) plate by agar well-diffusion technique. The MHA was prepared in double distilled water (pH 7.0) and sterilized in an autoclave at 121 ˚C for 15 min. Then, the sterilized MHA was poured into the petri plate and allowed to solidify at room temperature in laminar flow. Inoculum containing 106 cfu/mL of the freshly prepared bacterial culture was spread onto the MHA plates with a sterile cotton swab moistened with the suspension of the respective microbial culture. Then, three wells (9 mm in diameter) were punched into the MHA medium. 2 wells were filled with different concentrations (100 µg, and 50 µg) of the prepared hydrogel with the help of micropipette, and kept at room temperature for 4 h to allow diffuse compound into the medium. The third well was filled with erythromycin 5µg for streptococcus mutans and cephalexin 5µg for staphylococcus aureus as control drugs. The

culture plates were then incubated for 24 hours at 37 °C. Following incubation, each plate's zone of inhibition's diameter (mm) was noted. The results of the experiment were presented as the mean value and standard deviation (SD).

Mechanical Testing

Measurement of the Contact Angle

The water contact angle was calculated using EasyDrop wetting angle measuring equipment in order to assess the hydrophilicity of the scaffolds utilising the sessile drop method. On the surface of non-porous plane-parallel samples, the dosing device (Osilla goniometer) dripped roughly 5 uL of the generated hydrogel, and the contact angle was recorded after 2 s. In order to create the baseline, determine the contact angle, and interpret the results, drop shape analysis software was used.

Fourier transform infrared (FTIR) analysis

Fourier transform infrared (FTIR) spectroscopic analysis was performed using the Bruker alpha 2. The expected pendant functionalities of a agar-chitosan-rutin-carrageenan hydrogel were confirmed by the FTIR spectrum.

3. Results

Fig 1 - Antimicrobial Testing

Fig 1 - depicts the agar well disc used to characterize the antimicrobial activity

Fig 2 - Contact Angle Analysis

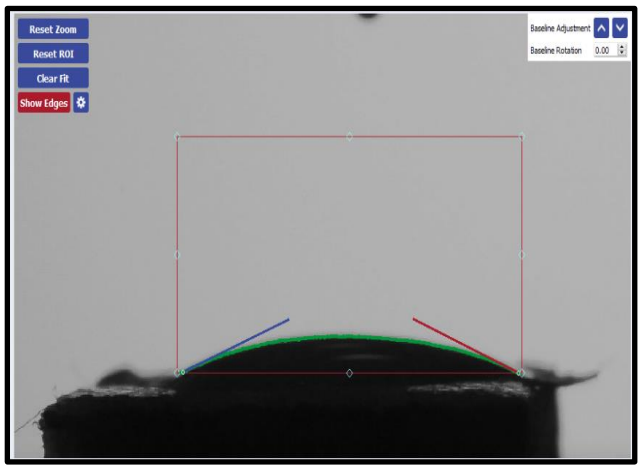

Fig 2 depicts that the prepared hydrogel was highly hydrophilic.

Fig 3 - Ftir Analysis

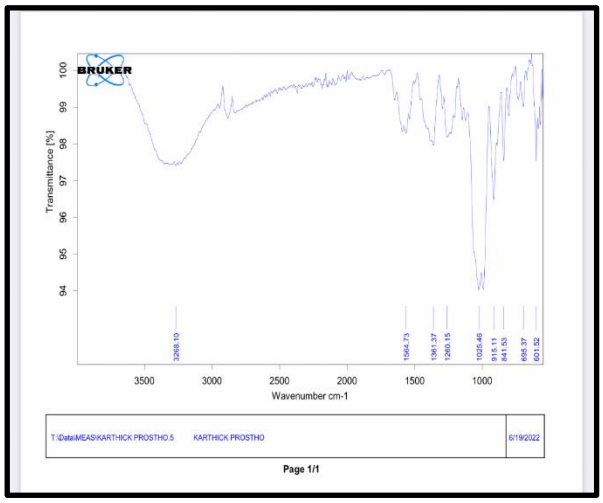

Fig 3 shows the functional group bond.

TABLE 1

Table 1 depicts the FTIR interpretation of the nature of functional group and bond present in the hydrogel preparation.

Value	Functional Group
3268.10	O-H stretch
1564.73	N- O stretch
1361.37	N- O stretch
1260.15	C-O stretch
1025.46	C-O stretch
915.11	O-H bend
841.53	C=C blend
695.37	Long chain methyl rock
601.52	C≡C—H

4. Discussion

Periodontal disease is the most common oral disease affecting the global population, resulting in pocket formation, clinical attachment loss, bone loss and tooth loss. Though various non-surgical and surgical treatment modalities and materials are available, complete and predictable periodontal regeneration is still elusive. Recently, application of tissue engineering principles for periodontal regeneration has shown great promise. Hydrogels have gained much attention in this regard, with potential for local drug delivery,wound healing and regenerative applications (20).

Hydrogels are natural, synthetic or composite polysaccharide-protein based polymeric biomaterials with excellent hydrophilicity. They make ideal substrates for wound healing, prolonged drug administration, bio-prosthetics, and endogenous regeneration of cells. Hydrogels with tissue mimicking microarchitecture and biochemical properties provide niche condition for cell survival, proliferation and differentiation (21,22).

Recently, hydrogel based on marine seaweeds have shown many biologic properties like antibacterial, antifungal, antioxidant, anti-

inflammatory properties. In the present study the hydrogel was prepared using seaweed biopolymers carrageenan, agar, chitosan and rutin. Carrageenan is a component of the cell wall and intercellular matrix of the seaweed tissues. It is a high molecular weight polysaccharide with an ester-sulfate content ranging from 15% to 40%. It is made up of D-galactose and 3.6 anhydro-galactose (3.6-AG) alternating units connected by 1,3 and 1,4 glycosidic linkages. Seaweeds of various types and species that belong to the Rodophyceae family are used to make carrageenan. Depending on the type of seaweed and the environmental factors at sea, such as light intensity, nutrient content, water temperature, and oxygenation, the carrageenan content of commercial seaweeds ranges from 20% to 40% of dry weight. Different forms of carrageenan, including kappa, iota, and lambda, are produced by seaweeds from various species and sources (23). Chitosan is the N-deacetylated version of chitin. Chitosan is an important biomaterial because of its inborn properties, which include its polyelectrolyte and cationic nature, mucoadhesion, hemostatic action, film-forming ability, biodegradability, and antibacterial activity. Chitosan is a versatile raw material that may be used to create 2D and 3D-based matrices, including membranes, fibres, particles, and composites, at the micro- and nanoscale for applications in TE of the skin, cartilage, and bone. Chitosan is frequently combined with other polymers (natural or synthetic) and/or proteins to create blends and composite materials. The production of chitosan derivatives has also involved a variety of other chemical and physical procedures.These chitosan-based materials are suitable for biomedical applications such as skin replacements, bone and nerve regeneration, cancer diagnostics, medication delivery, and gene therapy due to their suitable characteristics and functions (24). Chitosan and other macromolecules have been transformed into a range of bionanocomposites as a result of the advent of nanotechnology, with applications in regenerative medicine and drug delivery vesicles.
Agar is called hydrophilic colloid which is derived from specific Rhodophyceae seaweeds. Although it dissolves in hot water, it is insoluble in cold water. A 1.5% solution is transparent and solidifies into a gel at 34–43 °C. It does not melt again below 85 °C. It is a blend of polysaccharides, with galactose serving as the primary monomer. In comparison to carrageenan, these polysaccharides can be sulphated to extremely different degrees. Rutin is a rutinoside, which is quercetin with a sugar group substitution at position C-3 for the hydroxyl group. It functions as an antioxidant and metabolite(25). It is a rutinoside, a tetrahydroxyflavone, a quercetin O-glucoside, and a disaccharide derivative.(26) Rutin, a non-ionic polysaccharide, is a promising chemical because of the many uses it may be put to. In solid pharmaceutical formulations, it acts as a binder and a disintegrating agent; in liquid pharmaceutical formulations, it offers stabilisation and thickness(27).
When compared to the control medication in the current investigation, the hydrogel demonstrated good antibacterial action against the common oral germs Staphylococcus aureus, Streptococcus mutans and Candida Albicans. With an increase in hydrogel concentration, the zone of inhibition increased. This could be explained by the component polysaccharides chitosan, carrageenan and rutins antibacterial capabilities(28). The polysaccharides high sulfate content could have contributed to their antibacterial activity. In the literature, there are several instances of hydrogels based on carrageenan and chitosan that were created primarily as a remedy for wound healing and also Rutin and chitosan-based scaffolds have been proven to be effective biomaterials for bone regeneration(29). Studies have demonstrated the beneficial effects of hydrogels made from seaweed biopolymers in tissue engineering and periodontal therapy applications. The use of seaweed biopolymers hydrogels in the treatment of periodontal diseases merits further investigation.
Furthermore, the contact angle analysis proved that the prepared hydrogel had good wetting properties and would be highly hydrophilic that would be ideal for tissue engineering applications. Also , the FTIR analysis that was performed to analyse the functional groups present on the basis of frequency shift in FTIR. The formation of hydrogel based on carrageenan, chitosan, agar and rutin was fully supported.

5. Conclusion

The prepared hydrogel has good biocompatibility, hydrophilicity and promising antimicrobial properties. Further in vitro and in vivo studies should be conducted to explore its biologic interactions and potential for drug delivery and tissue engineering applications.

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Conflict Of Interest

No conflict of interest

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